Date: August 15, 2001

To: BLA STN 125029 file

From: Gibbes Johnson, Ph.D

Through: Amy Rosenberg, M.D., Barry Cherney, Ph.D.

Re: Drug Substance Review of BLA STN 125029, Activated Protein C (APC), Eli Lilly and Co.

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I. Drug Substance

I.A. Description, Including Physical and Chemical Characteristics and Stability of the Drug Substance

I.A.1. Nomenclature

International Non-proprietary Name (INN): drotrecogin alfa (activated)

Non-proprietary Name (USAN): drotrecogin alfa (activated)

Proprietary (Brand) Name: XIGRIS™

Synonyms: recombinant human Activated Protein C (rhAPC)

Lilly Compound Number: LY203638

Chemical Abstracts Service Number (CAS): 42617-41-4

Drug Substance is ---------- containing -- mg/ml rhAPC in -- mM citrate, --- mM NaCl, pH ---- stored at --- C.

I.A.1.a. Structural Formula

Molecular Formula: ---------- for heavy chain ---------- and light chain ----------, respectively.

(Protein backbone excluding ---------- portion)

Molecular Weight: ----------, and ---------- Daltons for heavy chain ---------- and light chain ---------- respectively.

(Protein backbone excluding ---------- portion)

Structural Formula: rhAPC is a ---chain glycoprotein containing ---------- amino acids for heavy chain ---------- and light chain ----------, ----------, respectively.

Recombinant human Activated Protein C (rhAPC) is a ---chain glycoprotein containing ------- N-glycosylation sites and --- disulfide bonds. The heavy chain contains ---- amino acids, in which ---------- residues are cysteine and --------- N-linked glycosylation sites (----------). The seven cysteine residues form -------disulfide bonds within the heavy chain and -------disulfide bond between the chains. The cDNA expresses a

--------amino acid light chain variant, but the major components found in rhAPC product are the ----------) amino acid residue light chain ---------- variants. The light chain contains -------N-linked glycosylation site (---------) and ----cysteine residues, which form ----- disulfide bonds within the light chain and ----- disulfide bond between the chains. The first ----- glutamic acids on the light chain are ————————————) and aspartic acid ----------. rhAPC has the identical amino acid sequence as human plasma-derived Activated Protein C. A representation of the primary structure of rhAPC is shown in Figure I.A.1.

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I.A.2. Confirmation of Structure

All physical and chemical data are in accord with the proposed structure for recombinant human Activated Protein C (rhAPC). The data in the following sections, unless otherwise noted, were from experiments using the primary reference standard ---------- The preparation of the primary reference standard is discussed in the Reference Standard section.

The structure of rhAPC has been established through various physicochemical techniques. ---------- results for the intact rhAPC, as well as the separated heavy and light chains of rhAPC, were consistent with the structure predicted from the gene sequence and expected post-translational modifications. ---------- analysis of the ---------- rhAPC standard, as well as the results of ———————————— content analysis and ----------, indicated that the first -------- glutamic acid residues in the N-terminal region of the light chain were fully γ-carboxylated, as expected. ---------- characterization of all significant peaks in a ---------- of the ----------rhAPC standard provided confirmation of the expected amino acid sequence, and also indicated that amino acid residue ---------- was fully ————————. The rhAPC standard was demonstrated to consist of a mixture of light chain C-terminal variants terminating at amino acid residues ----------, based on ---------- results. These data, in combination with the ---------- data for the expression construct, provide conclusive evidence that rhAPC reference standard Lot ---------- has the expected amino acid sequence.

The ---------- structures present at each site were confirmed by ---------- analysis, as well as by comparison to the structures deduced from ---------- linkage analysis for an earlier recombinant human Protein C (rhPC) development lot. ---------- chromatography with ---------- (----------) analysis of the ---------- confirmed that the profile for rhAPC reference standard Lot ---------- was similar to the that of the earlier developmental lot (----------). Hence, structural data obtained by -------- and --------- linkage analysis for the developmental lot can appropriately be used to deduce ---------- structures present in the rhAPC reference standard. The data

demonstrate that rhAPC is N-glycosylated at ----------, and ----------, whereas the ---------- sites are approximately ----------, respectively.

---------- analysis for ---------- peptides or ---------- peptides obtained from various ---------- of intact rhAPC standard demonstrated that all --- cysteine residues formed -- expected disulfide bonds.

The higher order structure and thermal characteristics of rhAPC reference standards were evaluated using ----------

The structures of ------rhAPC **full-scale consistency lots**----------, were characterized using ---------- for protein variants. The results from ---------- are shown in Section I.C.3.d.5., Comparability of Drug Substance Manufactured at Pilot Scale and Commercial Scale. This section provides the results from ----------, and molecular weights of the predominate heavy (----------) and light chain (-------) components determined by ----------. The data demonstrate that the structures of each of the ---- full-scale consistency lots, ----------, of rhAPC drug substance were consistent with the rhAPC primary reference standard, Lot --------.

I.A.2.b. Expression of Strength

The strength (quantity) of recombinant human Activated Protein C (rhAPC) drug substance is expressed as mg/mL, determined using a ---------- assay that measures rhAPC protein content. The rhAPC drug product is labeled as mg/vial, also determined using the ---------- assay.

The antithrombotic activity, or potency, of each rhAPC ---------- and drug product lot is determined using an ----------) assay. This assay is performed to confirm that the specification limit for potency is met for each rhAPC lot. The potency value is expressed as units/mg. Since an international reference standard of activated protein C is not available, potency units are defined relative to an in-house reference standard. The initial in-house reference standard, Lot --------, was assigned a potency of ----- units/mg, based on the following logic:

[

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The potency of the subsequent in-house primary reference standard, Lot --------, was determined to be ---- units/mg by direct comparison to reference standard Lot -------- using the ------ assay. The potency of subsequent reference standards will be established by direct comparison to the in-house primary reference standard, --------. If an international reference standard for purified aPC is established in the future, the potency for rhAPC will be redefined in terms of international units/mg by comparison to the international reference standard.

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I.B. Manufacturer of the Drug Substance

I.B.1. Name and Addresses of the Manufacturers

The names and addresses provided in this section include the following contract facilities: ----------. Eli Lilly and Company has entered into contractual and technical agreements with these firms for the production and control of

recombinant human Activated Protein C (rhAPC) drug substance. The agreements define the responsibilities for each provider. Lilly's Quality Unit and the local site Quality Unit assure that each contract facility complies with the predetermined agreements provided for in the contracts, as well as cGMPs per 21 CFR§210 and 211, and the requirements of 21 CFR§600, 601 and 610. ----------were utilized only for the preparation and control of the original master and working cell banks. Additional detail regarding the responsibilities for the contract manufacturer, ----------., is provided in Section I.B.1.a., Contract Manufacturer Responsibilities.

Master and Cell Bank Facilities

The master cell bank (MCB) and working cell bank (WCB) were prepared by:

[

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Viral safety and adventitious agent testing of the MCB and WCB were performed by:

[

]

Characterization testing of the MCB and WCB was performed by:

[

]

And

[

]

Manufacturing Facilities

The bulk rhAPC drug substance, including cell culture and harvest, recovery, and purification, will be performed at:

[

]

The bulk rhAPC drug substance will be stored at:

Eli Lilly and Company
Lilly Materials Center
Indianapolis, Indiana 46285-0002
USA

Control Facilities

The adventitious agent testing of the MCB and WCB will be performed at:

[

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The adventitious agent testing of ---------- rhAPC will be performed at:

[

]

and/or

[

]

In-process testing of rhAPC will be performed at:

[

]

Lot release testing and batch release of the rhAPC drug substance will be perfo rmed at:

[

]

Final Quality Control release of the drug substance will be performed by:

Eli Lilly and Company

Indianapolis, Indiana 46285-0002
USA

Stablity testing of the rhAPC drug substance will be performed at:

Eli Lilly and Company

Indianapolis, Indiana 46285-0002
USA

And

Eli Lilly and Company

Indianapolis, Indiana 46285-0002
USA

I.B.1.a. Contract Manufacturer Responsibilities

---------- was responsible for preparation and testing (consisting of growth, productivity, stability of production) of the master and working cell banks from a cell line cloned by Eli Lilly and Company.
---------- is responsible for: (1) receipt and testing of raw materials for use in the manufacture of rhAPC drug substance, (2) cell culture to produce the precursor molecule, (3) purification and activation to the active molecule, and (4) final purification and ----------of the drug substance. -------is responsible for all in-process intermediate and final release testing of each lot of rhAPC drug substance (with the exception of testing for viruses and adventitious agents as noted below), and for release of rhAPC drug substance lots to Lilly.

Eli Lilly and Company (the license holder) is responsible for final release of rhAPC drug substance lots and for ongoing stability testing of rhAPC.

Assuring Compliance of the Contract Manufacturer

Lilly has prepared a Manufacturing Responsibilities Document with the contract manufacturer, ----------, which specifies the responsibilities of each party, and the review and control of those responsibilities. This document has been approved by both parties. Process control documents have been jointly developed. Lilly may review manufacturing batch records and other master control documents to be used by ----------. Process changes and deviations, while the responsibility of -------to prepare and document respectively per their internal procedures, are subject to review and appropriate approval by Lilly. Lot release results generated by -------are reviewed for each lot prior to final release of the lot by Lilly for further manufacture. Regular meetings are held between both parties for review of manufacturing and analytical results, to ensure process control. A yearly assessment of product quality is jointly prepared. Regular audits by Lilly are done of the manufacturing facility to ensure compliance.
Results from the contract testing laboratories are reviewed by Lilly, and the contract testing laboratories are audited on a regular basis.

I.B.3. Additional Products in Manufacturing Facility

----------operates a multi-product facility at its ---------- ----- manufacturing site. The areas used during the production of recombinant human Activated Protein C (rhAPC) have been segregated from the processing of other products, though some support functions are multi-product. Extensive measures are, therefore, taken to prevent potential cross contamination and mixup of materials, product, and equipment. These are described in detail in Section I.B.4., Precautions Taken to Prevent Contamination.
Information concerning other products manufactured at the ---------- facility

are contained in -------Drug Master File ---------- A Letter of Authorization allowing Eli Lilly and Company to reference ---------- is provided.

I.B.4. Precautions Taken to Prevent Contamination

Overview

Recombinant human Activated Protein C (rhAPC) is manufactured in ---------- multi-product facility at its ---------- site. The drug substance, rhAPC, is manufactured in dedicated cell culture and purification suites. Media preparation, buffer preparation, raw material dispensing, and small equipment washing/autoclaving are performed in non-dedicated, multi-product areas. Raw material dispensing and media preparation are done for a single process at a time, with area cleaning between products. Small equipment and glassware from each process are decontaminated using a validated method, prior to washing in the automated washer. The following sections present details of the facility and equipment design and the procedural methods employed to prevent the possibility of contamination or cross contamination of cell lines or products.

Facility and Equipment Cleaning/Disinfecting Regime

Manufacturing areas are cleaned and disinfected on a regular schedule in order to minimize the potential for contamination of products by the removal of microbial and particulate contaminants from the process environment. Disinfecting agents are chosen for their ability to prevent development of resistant organisms and have been validated against routine flora found in the facility.

Surfaces in the manufacturing facility have been designed to permit ease of cleaning and are smooth, non shedding and free from cracks and open joints. Pipework, light fittings, ventilation points and other services have been designed and located in such a way as to avoid the creation of recesses and hard-to-clean surfaces. Floor drains are designed to be free draining and are sanitized with ---------- on a routine basis.

Details of the cleaning/disinfecting regimes for the operational areas are available at ---------- facility.

Most manufacturing processing equipment is designed to be ----------

-------- sequences are validated. Cleaning validation includes ----------.

Separate ------- systems are used for cell culture/primary recovery (pre-Viral Inactivation) and purification (post-Viral Inactivation).

Some equipment, such as chromatography columns and systems, and ---------- or ---------- rigs are designed to be ----------.

Most manufacturing processing equipment is designed to be ---------- as needed.

All ---------- procedures are validated by ----------

Some equipment, such as chromatography columns and skids, ---------- and ---------- rigs are ----------

All critical filters in the cell culture suite are ---------- --------- after use. The ---------- ---------- are ---------- following use.

Details of the cleaning and sterilization validation policy, and performance qualification validation protocols for all types of equipment, are available at ----------

I.B.4.c. In Process Controls to Prevent Contamination

Raw Materials

All raw materials used in the manufacture of rhAPC are purchased from approved suppliers. Written specifications exist for all raw materials describing acceptance and release criteria. Raw materials are inspected and checked against these specifications by Quality control personnel before being released for use in the manufacturing facility. If raw materials need to be purchased from alternative suppliers to those currently approved, the raw material will be required to be of similar quality standard and have similar accompanying quality information as the existing supply. Raw materials of animal origin are subject to extensive testing for ---------- before acceptance by ----------. Full details of these materials and all others used in the

manufacture of rhAPC are provided in Section I.C.1., Specifications for Raw Materials Used in the Manufacture of the Drug Substance.

In-Process Testing

In-process product samples are taken throughout each manufacturing run to ensure that product quality and integrity are maintained. The testing regime is provided in Section I.D.1., In-Process Controls.

All medias and buffers used in the production of rhAPC are filtered and tested for ----------

Antifoam solution used in the cell culture of rhAPC is autoclaved.

Regular checks for the absence of microbial contamination are made during the ---------- ----------stages of production. The reactor is tested for the presence of ---------- at the end of the cell culture.

---------- micron (or smaller) filters are used at multiple processing steps to minimize ----------

---------- levels are determined throughout the purification process at all critical steps including:

[

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Results are reported to quality and manufacturing management, and handled per the appropriate SOP. The cleanliness and, where appropriate, sterility of equipment used during the manufacture of rhAPC, is ascertained prior to use as previously described in this section under “Vessel Cleaning and Sterilization.”

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I.C.2.b. Lot Definition

Details of the operation are described in Section I.C.3.d.2., Flow Diagram for Purification Process with Critical Process Parameters and Criteria for Forward Processing, and Section I.C.3.d.3., Description of the Purification Process.

Following ---------- (refer to Section I.C.2.a., Process Flow Diagram – Overview, Process Step 7), the ----------(up to ----------). The column is eluted within --- hours of ----------

[

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---------- The scale of these operations is defined by the critical process parameters for the individual steps. In largest part, the maximum lot size is limited by the ----------). The minimum lot size is bounded by the ----------). The eluate from the chromatography column is diluted to approximately --- grams per liter, sampled, and ---------. (The process steps defining a lot of drug substance are provided in the bold box in Figure I.C.1 below. The process step numbers correspond to those of Section I.C.2.a., Process Flow Diagram – Overview)

[

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I.C.3.d. Purification and Downstream Processing

I.C.3.d.1. Definition of Batch

The product of the purification process ----------, therefore, the definition of a purification batch is identical to the definition of a lot. The definition of a lot is provided in Section I.C.2.b., Lot Definition.

Details of the downstream processing are described in Section I.C.3.d.2., Flow Diagram for Purification Process with Critical Process Parameters and Criteria for Forward Processing, and Section I.C.3.d.3., Description of the Purification Process.

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Column Lifetime

Studies have confirmed that resin subjected to --- cycles generates a mainstream that meets all of the criteria for forward processing and a reproducible chromatographic profile. ---------- confirm the absence of product in the mainstream elution fraction, demonstrating suitability of cleaning. Viral clearance has been shown to be unaffected by up to --- elution cycles.

This has taken from I.D.1.b (In-process controls for purification):

Column Lifetime and Resin Reuse

Resin from a column subjected to --- cycles showed comparable performance at the laboratory scale to chromatography on new resin. ---- cycles exceeding --- runs were

demonstrated at the pilot scale in the production of clinical trial material. Another cycle of --- runs was demonstrated at the commercial scale in a development facility. Each of these systems generated mainstreams that met all of the criteria for forward processing, a reproducible ----------, and no significant changes in ---------- ---------- Resin subjected to --- cycles at the commercial scale was used in viral clearance studies and gave rise to the same levels of viral clearance as new resin.
Suitability of the cleaning regimen is demonstrated by the ----------, ----------. Viral inactivation by the regeneration solutions is discussed in the viral safety assessment (Section I.D.3., Verification of Viral Safety). Resin subjected to --- cycles and ---------- ---------- confirmed the absence of product in the mainstream elution fraction.
Suitability of resin reuse will also be confirmed in the manufacturing facility. At the end of the consistency runs and after --- cycles, commercial columns will be loaded with a ---------- and the mainstream fraction will be assayed for ---------- ---------- ---------- The simulated mainstream fraction will also be subjected to SDS-PAGE analysis.

REVIEWER'S COMMENT: IS --- CYCLES THE DEFINED LIFESPAN FOR THE COLUMN?

ANSWER: YES. THIS WAS CONFIRMED AND DOCUMENTED BY FRED MILLS DURING THE -------INSPECTION IN JUNE, 2001

Storage of Intermediate

The mainstream is held at --------- in an ultra ----------. Hold time is not to exceed --- days. Material held under these conditions has been shown to meet or exceed all criteria for forward processing for the duration of this period. The ------ meet the Ph.Eur. and USP criteria for ----------

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Equipment

Retentate vessel with ----------

---------- Filters (----------

Support vessels (----------)

---------- ---------- Control Skid

Critical Process Parameters

1. ----------

Criteria for Forward Processing

1. ----------

Storage of Intermediate

Retentate is held in ---------- vessel at (----------) for no more than --- hrs.

These hold times have been demonstrated to be acceptable by ----------

---

REVIEWER'S COMMENT: HAS A MAXIMUM NUMBER OF CYCLES BEEN DETERMINED FOR THE COMMERCIAL SCALE MEMBRANE? HAS THE

ABILITY TO CLEAN THE MEMBRANE BEEN VALIDATED THROUGH THIS LIFESPAN?

ANSWER: NO and NO. FRED MILLS FOLLOWED UP ON THIS AT THE ------- INSPECTION. THE SPONSOR NEEDS TO ESTABLISH A PROSPECTIVE PLAN TO ADDRESS THIS ISSUE.

Step 10 - Activation of Recombinant Human Protein C with Thrombin

Purpose

The purpose of this step is to enzymatically convert protein C zymogen to rhAPC by removal of the activation peptide with thrombin.

Step Description

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Equipment

---------- reaction vessel (----------)

Support vessels (----------)

Critical Process Parameters

[

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Criteria for Forward Processing

Storage of Intermediate

Activated protein C ----------. Aggregate processing time between the quench of the activation reaction and reaching ----C in the ---------- will not exceed ------- hours. This processing time limit is supported ---------- analysis of isoforms and

degradation products.

Step 11 - ---------- (----------)
Purpose

The purpose of this step is to provide additional assurance of the control and clearance of potentially contaminating viruses.

Step Description

[

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Storage of Intermediate

Activated protein C ----------. Aggregate processing time between the quench of the activation reaction and reaching ---C in the ---------- will not exceed ---- hours. This processing time limit is supported by ---------- analysis of isoforms and degradation products.

REVIEWER'S COMMENT: FRED MILLS CONFIRMED AT THE ------- INSPECTION THAT THIS MEMBRANE IS ----------

Step 12 - ---------- Chromatography

Purpose

The purpose of this step is to concentrate the rhAPC, to purify it away from process specific contaminants such as thrombin, and to exchange the protein into a matrix compatible with formulation operations. This step is also a part of the process viral clearance capability.

Step Description

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The ---------- column is unpacked and the resin is ----------

Equipment

Charge and buffer containers (either ---------- tanks or ----------)

Chromatography Column (----------)

Control and Monitoring Chromatography Skid (---------- product contact surfaces)

Mainstream collection vessel (----------)

Critical Process Parameters

[

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Criteria for Forward Processing

Mainstream concentration (7.5 - 12.5 grams of rhAPC per liter by A280).

Column Lifetime

Studies have confirmed that ---------- resin subjected to --- elution cycles generates a mainstream that meets all of the criteria for forward processing and a reproducible chromatographic profile. ---------- have been shown to be acceptable through --- elution cycles. ---------- over used resin confirm the absence of product in the mainstream elution fraction, demonstrating suitability of cleaning. Viral clearance has been demonstrated to be unaffected by up to --- elution cycles.

Taken from page 510 (I.D.1.b In-Process Controls for Purification):

Column Lifetime and Resin Reuse

The ---------- column is ----------.

Studies have confirmed that ---------- resin subjected to --- elution cycles generates a mainstream that meets all of the criteria for forward processing and a reproducible chromatographic profile (**reviewer's note: this is lab scale**). ---------- have been shown to be acceptable through --- elution cycles. ----------) over used resin confirm the absence of product in the mainstream elution fraction, demonstrating suitability of cleaning. Viral clearance has been demonstrated to be unaffected by up to --- elution cycles. Suitability of resin reuse will also be confirmed in the manufacturing facility. At the end of the consistency runs and after --- cycles, commercial columns will be ----------.

REVIEWER'S COMMENT: IS --- CYCLES THE DEFINED LIFESPAN FOR THE COLUMN?

ANSWER: THIS IS NOT EXACTLY CLEAR. FRED MILLS WAS GIVEN AN ANCILLARY CLEANING PROTOCOL, WHILE AT THE ---------- INSPECTION, WHICH INCLUDED THE ANALYSIS DESCRIBED ABOVE AFTER ---------- CYCLES.

Storage of Intermediate

Activated protein C ----------. Mainstream fraction should be diluted to --- g/L in less than - hours. Aggregate processing time between the quench of the activation reaction and reaching ---C in the ---------- will not exceed ---- hours. This processing time limit is supported by ---------- analysis of isoforms and degradation products.

Step 13 ---------- of the rhAPC

Purpose

The purpose of this step is to --------- the rhAPC drug substance for storage and shipment.

Step Description

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Equipment

Critical Process Parameters

[

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Criteria for Forward Processing

1. Release specifications for the drug substance (Section I.F.1., Drug Substance Specifications and Tests).

Storage of Intermediate

Activated protein C ----------. Aggregate processing time between the quench of the activation reaction and reaching ---C in the ---------- will not exceed ---- hours. This processing time limit is supported ---------- analysis of isoforms and degradation products.

Storage conditions and stability of the BDS are described in Section I.H., Stability of the Drug Substance.

I.C.3.d.4. Comparison Between Pilot Scale and Commercial Scale Manufacture

Chromatography

For both chromatographic steps, all Critical Process Parameters and Criteria for Forward Processing are identical in pilot and commercial scale operations. The composition and specifications for buffers and chromatographic matrices are the same at the two scales, as

well as ----------. The only differences in the chromatographic operations are in the column ----------, which do not have a significant impact on column performance given a uniform packing. ----------

---

---------- Filtration

Membranes used in commercial operations are from the same vendor and have the same specifications as those used at the pilot scales. Membranes were prepared and suitability for use was confirmed in the same manner at both scales, with very slight differences being associated with the hydrodynamics of the equipment used. All Critical Process Parameters and Criteria for Forward Processing are the same at the pilot and commercial scales. Preparation and composition of the processing solutions are the same at both scales, and volumes have been linearly scaled. The slight differences (---%) in both the inlet and outlet pressures of pilot scale and commercial scale operations are a function of differences in the hydrodynamics of the skids and the pumps used at the two scales.

Activation with Thrombin

All Critical Process Parameters and Criteria for Forward Processing are the same for operations at the pilot and commercial scales. Pilot scale operations used thrombin supplied from both of the vendors identified to supply the commercial operations; thrombin specifications were the same for pilot scale and commercial scale operations. There are no differences in activation time, temperature, duration, or concentration of reactants at the commercial and pilot scales. There were no differences in the reaction kinetics or the isoform profiles at the commercial and pilot scales.

The same membranes and membrane suitability tests were used in the pilot and commercial scales. ---------- was performed at the pilot scale using ----------, while commercial operations will be executed using a ---------- unit. Consequently, there are differences in the

---

---------- The values used in commercial operations either represent comparable hydrodynamics to pilot and lab scale validation studies or have been developed to make small scale viral clearance studies "worst case" with respect to viral clearance.

Controlled ---------- Operations

Both pilot and commercial scale operations execute controlled ---------- operations using ---------- of identical design and materials of composition, except for total volume ---------- cycles are similar at both scales. Characterization of material ---------- at the pilot and commercial scales is provided in Section I.H., Stability of the Drug Substance.

I.C.3.d.5. Comparability of Drug Substance Manufactured at Pilot Scale and Commercial Scale

During the clinical development program several process modifications were implemented, as summarized in Table I.C.22. Preclinical as well as Phase 1 and 2 clinical trial lots were manufactured using a development process designated ----------process the purified drug substance manufacturing solution ----------. Subsequent optimization studies led to the development of the initial commercial process (--------) which was used to manufacture lots for Phase 3 clinical trials. The -------- process provided improved stability for the drug substance manufacturing solution, ----------. In addition, ---------- led to more uniform product quality for the -------- process, compared to --------. In changing from the -------- to the -------- process, a ---------- step was added to provide a greater level of viral safety assurance and the animal-source raw materials ---------- were removed. **During Phase 3 a slightly modified commercial process (---------) was introduced. The --------- process used ----------**

----------. The drug product formulation used throughout Phase 3 (i.e., for both the -------- and --------- lots) was the commercial formulation (------).

Comparability Data for Pilot-Scale Processes used During Clinical Development

Batch analysis data for pilot-scale clinical trial lots produced by the initial commercial process (--------) and the commercial process (---------) are provided in Section I.F.2., Tabulation of Analytical Data on Drug Substance Lots. Batch analysis data for drug product lots produced by the -------- process are provided in Section II.E.2.c., Tabulation of Analytical Data on rhAPC Drug Product Lots. A summary of the range of assay values obtained for pilot-scale lots produced by the --------, -------- and --------- manufacturing processes is shown in Table I.C.23. In general, the assay values observed for -------- material were comparable to those observed using the later processes. However, variability for some parameters (e.g. potency, ----------) tended to be considerably greater for the -------- process. The --------- levels observed for most of the -------- lots was below the assay detection limit of ---------- lots contained levels in the range of ------ppm. However, all lots produced using the -------- and --------- processes met the ---------- specification of not more than -- ppm in place throughout the clinical development program.

Reviewer's note: Thrombin is -------. See Fred Mills review for more information

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Comparison of Full-scale and Pilot-scale Drug Substance Lots

Ten full-scale lots produced at the commercial facility have been characterized using the specification assays as well as additional characterization assays and the product quality was compared to that of the pilot-scale lots used in Phase 3. A summary of the data obtained is provided in Table I.D.24 and batch analysis data for each individual lot are provided in Section I.F.2., Tabulation of Analytical Data on Drug Substance Lots. Table I.D.25 provides additional information concerning the usage and processing history for the ---- full-scale lots included in this data summary. ---- of the full-scale drug substance lots, derived from ----- different bioreactor runs were designated as validation lots. ------ lots of drug substance, from ----- different bioreactor runs were used to produce ----- drug product validation lots (------ of each presentation). Batch analysis data for the full-scale drug product validation lots (Section II.E.2.c., Tabulation of Analytical Data on rhAPC Drug Product Lots) demonstrate that the commercial drug substance is suitable for

manufacturing commercial drug product that consistently meets specifications and is of comparable quality to lots used in Phase 3 clinical trials.

The data provided in Table I.C.24 show that the full-scale drug substance meets the proposed specifications and that the product quality of the full-scale and pilot-scale lots is comparable. In addition to the specification assays, a comprehensive and diverse battery of additional characterization assays were performed to assess the structural integrity and comparability of the full-scale validation lots. ---------- analysis were performed on the ---- drug substance validation lots and data are provided in Section I.A.2., Confirmation of Structure. These data are in accord with the expected protein structure, including the expected post-translational modifications; ----------. As shown in Table I.C.24, direct --- content analysis provided additional confirmation that ---------- was complete. Additional confirmation of the protein structure is provided from the ----------, shown in Figure I.C.26 through Figure I.C.28 (---------- is shown in Figure I.C.25). All lots met the assay criteria for identity described within the analytical methods.

---------- was confirmed by ---------- analysis (shown in Table I.C.24) as well as by ---------- of the ---------- as shown in Figure I.C.30 through Figure I.C.34 (see Figure I.C.29 for the ----------). These data demonstrate that the ---------- for the full-scale validation lots are comparable to that of the primary reference standard --------. All lots met the ---------- criteria specified in the assay procedure. As shown in Table I.C.24 the overall ----------, as well as the ---------- of the full-scale lots were comparable, within the variability of the assay, to that of the pilot-scale lots. ---------- content is a key indicator of ---------- control, since bioreactor conditions can affect both the extent of ---------- and the levels of ---------- leading to ---------- in the harvest stream. As shown in Figure I.C.35 and Table I.C.24, ---------- content, as well as overall ----------, for the full-scale lots was well-controlled and comparable to that of the pilot-scale lots.

As shown in Table I.C.24 and Figure I.C.36 potencies of the full-scale lots were

comparable to that of the pilot-scale lots. The mean value for the full-scale lots was approximately ---- units/mg compared to approximately ---- units/mg for the pilot-scale -------- and --------- lots (-------- lots tended to have lower and more variable potencies). The difference in mean potency between the pilot-scale and full-scale lots is approximately --%, or approximately ---------- for the assay. This difference is not of practical significance and largely represents assay variation (within laboratory as well as between laboratories), rather than process variability. These data clearly demonstrate that the full-scale process consistently produces drug substance lots having potencies within the range observed for Phase 3 clinical trial lots.

Key purity assay parameters include ---------- The data provided in Table I.C.24, as well as Figure I.C.37 through Figure I.C.39 demonstrate that the purity of full-scale drug substance lots consistently conforms with the proposed specifications and is comparable to material produced at pilot-scale and used in Phase 3 clinical trials. Purity profiles for the ---------- assays are shown in Figure I.C.40 through Figure I.C.54 (example ----------assays are provided in Figure I.C.40 and Figure I.C.49, respectively). Profiles from two additional identity assays---------- are presented in Figure I.C.55 through Figure I.C.63. ---------- profiles for representative pilot-scale drug substance are shown in Section I.D.2.b.1., Identification of Potential Impurities. No significant levels of new related substances were observed for the full-scale lots compared with the pilot-scale lots used in clinical trials. As shown in Table I.C.24 the mean level of ----------was slightly higher (------%) compared with pilot-scale lots, however this difference is relatively small and all individual assay results are within the specification limit (not more than 0.1%).

As shown in Table I.C.24, ---------- determined by ---------- were comparable for the full-scale and pilot lots. The slightly higher level of ---------- observed for the full-scale lots is consistent with the observation of a slightly elevated level of light ----------(determined by

the ---------- assay) and may reflect a slightly higher degree of ---------- for the full-scale process. However, these differences are small and do not represent a significant change in purity. As shown in Table I.C.24 and Figure I.C.64 and Figure I.C.65 levels of the ----------were comparable for the full-scale lots and lots produced by the -------- and -------- processes. Variability of the ---------- profile was significantly greater for the -------- process. These data demonstrate that the full-scale process produces a product having a very reproducible ---------- that is comparable to the profile observed for pilot-scale material used in Phase 3 clinical trials.

Data shown for additional assays on full-scale lots shown in Table I.C.24 indicate that the levels ---------- are well-controlled and that the solution ---------- are within the specification ranges. No DNA was detected for any of the drug substance lots above the assay detection limit of ----------. As expected the ---------- content of drug substance lots produced by the -------- process was approximately ---- fold higher than for lots produced by the commercial process, owing to ---------- concentrations for lots from the pilot-scale (--------) and full-scale commercial processes were comparable.

The production process ---------- operating over approximately a -- day period. Details concerning ---------- used to produce each drug substance lot are provided in Table I.C.25. Examination of data provided earlier in this section for the full-scale lots reveals no apparent effect of these parameters on the ---------- for the full-scale lots. In addition, no significant differences in ---------- are observed. Potential effects of ---------- on drug substance --------, ---------- ----------) were further assessed, as shown in Figures I.C.66 through Figure I.C.71. These data indicate that there is no significant impact of ----------

------- identity on product quality.

Conclusions

This detailed physicochemical and *in-vitro* biological assessment of rhAPC drug substance demonstates that material produced by the full-scale commercial process consistently meets specifications and is comparable to material produced at pilot-scale and used in clinical trials. In addition, the full-scale rhAPC drug substance has been used to produce commercial-scale drug product lots that meet specifications. Finally, there is no significant impact of ----------) on the quality of the full-scale rhAPC drug substance lots.

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I.D. Process Controls

I.D.1. In-Process Controls

I.D.1.a. In-Process Controls for Bioreactor and Recovery Steps

The in-process controls for the cell growth, harvest, and initial recovery are of two types: the control of critical process parameters during the process, and criteria for forward processing (specifications) for designated steps of the process. Table I.D.1 provides an overview of the in-process criteria for forward processing for cell growth, harvest, and initial recovery. The rationale for the process control parameters, the Critical Process Parameters (CPP) and the Criteria for Forward Processing (CFP) is described for each step. An overview over the process is provided in Section I.C.2.a., Process Flow Diagram – Overview. The flow diagram from that section is shown below.

I.D.1.b. In-Process Controls for Purification

The in-process controls for the purification of the drug substance are of two types: critical process parameters and criteria for forward processing (in-process specifications). Critical process parameters are listed in Section I.C.3.d.2., Flow Diagram for Purification Process with Critical Process Parameters and Criteria for Forward Processing, and Section I.C.3.d.3., Description of the Purification Process. Critical process parameters are control elements that are linked either to the achievement of the purpose of the step or to the prevention of an event deleterious to downstream processing. A deviation from the critical process parameters will trigger an investigation ----------

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---------- ----------) in compliance with cGMPs and standard operating procedures. Critical process parameters also provide linkage between representative laboratory scale and pilot scale operations and commercial scale operations.

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Ranges are generated from either laboratory or pilot scale studies as noted. They are all consistent with process ranges used in the manufacture of clinical trial material. Completed batch records and validation reports confirm the ability of commercial manufacture to comply with all controls and ranges described on pages 495-512.

I.D.2. Process Validation

The process validation has been successfully completed and resulting data reviewed. All consistency runs were performed in compliance with established cGMPs and with approved validation protocols. All excursions from the validation protocol, which includes the Criteria for Forward Processing (CFP) and Critical Process Parameters (CPP), were thoroughly investigated, as required by the validation protocol, and determined to have no impact on the validity of the consistency runs. Reports are available at the ----------, ----------, facility.

Reviewer's note: The validation protocol was submitted to the BLA as Amendment 8.

I.D.2.b. Validation of Purification Process

I.D.2.b.1. Identification of Potential Impurities

The following sections provide tabulation of potential impurities arising from the drug substance manufacturing process. Removal of the potential impurities described in this section are provided in Section I.D.2.b.2., Removal of Impurities During the Drug Substance Purification.

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I.E. Reference Standard

I.E.1. Primary Reference Standard

Reference Standard	Use
rhAPC Reference Standard History	
rhAPC Reference Standard, Lot --------	rhAPC Reference Standard
hPC Reference Standard, Lot ----------	hPC Reference Standard
---------- Thrombin Reference Standard, Lot---------	Thrombin Reference Standard
---------- Reference Standard, Lot ----------	Host Cell Protein (HCP) Reference Standard
Analytical Methods Used for Characterization of Reference Standards	

Characterization results and the supporting documentation are supplied for the reference standards listed above. Lot -------- is the primary recombinant human activated protein C (rhAPC) Reference Standard. Lot ---------- is the human protein C zymogen (hPC) primary reference standard. Lot --------- is the reference standard used to support the determination of ---------- in rhAPC drug substance, and -------- is the reference standard used to support the determination of ---------- in rhAPC drug substance..

rhAPC Reference Standard History

An rhAPC solution (Lot ---------- produced using an early development manufacturing process, was used to produce the first (preliminary) corporate rhAPC reference standard (Lot ----------). Aliquots of this solution corresponding to approximately -- mg/vial of rhAPC protein were dispensed ---------- vials, lyophilized, and sealed with ---------- stoppers. Lot ---------- was used as a reference

standard for characterization of early development lots of rhAPC.

-------- (----------

A single rhAPC process solution (Lot ----------), produced using the manufacturing process that was used to produce toxicology and Phase 1 and 2 clinical trial lots, was used to manufacture two rhAPC vial-lyophilized lots. To produce the first lot, --------, the process solution was diluted with --------- buffer to a concentration of approximately -- mg/mL rhAPC. ----- mL aliquots of this solution were dispensed into -- mL ---------- vials, lyophilized, and sealed with -------- stoppers. The second lot, ----------, was produced in a similar manner, except that the drug substance was diluted to an rhAPC concentration of approximately -- mg/mL, and - mL aliquots of this solution were dispensed into ---------- vials. Lot -------- was used as the reference standard for all quantitative assays that required use of a reference standard, whereas ---------- was used as the control for certain identity assays (e.g., ----------

----

Lot -------- was established as a primary reference standard in 1999. It was prepared to provide a reference standard derived from rhAPC drug substance produced using the commercial cell bank and manufacturing process. The potency (determined using the ------ ---------- bioassay) was established using the previous in-house reference standard, Lot --------, as the assay standard.

Lot -------- was manufactured from rhAPC drug substance **Lot** ----------. The solution composition was adjusted to approximately -- mg/mL rhAPC, ----------, and 10mM citrate buffer, pH 6.0. One-milliliter aliquots of this solution were dispensed into -- mL ---------- vials, lyophilized, and sealed with -------- stoppers.

To ensure long-term availability of this material for use as a primary reference standard, a portion of the vials were segregated and designated Lot --------. The remaining vials were made available for use as a working standard. All rhAPC reference standards were stored at ---C or below.

REFERENCE STANDARD PROFILE

Name: Recombinant Human Activated Protein C (rhAPC)

Lot Number: --------

Defined Potency: ---- mg rhAPC protein/vial (excluding ----------). --- units/mg, for ------ ----------g assay. DO NOT WEIGH. Reconstitute entire contents of vial.

Handling: Normal laboratory precautions for recombinant products should be followed.

Storage: -------mg rhAPC protein (excluding ----------) lyophilized per flint glass vial with -------- stopper and ---------- flip-cap stored at ---------- temperature, ---°C to --------°C.

Lot -------- will serve as the primary rhAPC reference standard, and future standards will be compared directly to this lot.

I.F. Specifications and Analytical Methods

I.F.1. Drug Substance Specifications and Tests

The specifications for rhAPC drug substance have been established on the basis of historical experience with the manufacture of this material by Eli Lilly and Company and by ---------- In particular, they are based on the quality of rhAPC used in toxicological and clinical testing and in development of the drug product. The stability of rhAPC drug substance and the expected variability of the analytical methods have also been considered in establishing specifications. These specifications assure the quality standards of the drug substance at release and throughout the re-test period. The analytical methods and validations are provided in Section I.F.3., Analytical Methods and Validations for rhAPC Drug Substance.

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Reviewer's note: specification for ---------- is NMT --EU/mg (see amendment 11 dated June 11, 2001)

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I.F.1.a. Rationale for the Specifications and Tests Performed

Tests and specifications for rhAPC drug substance have been established in accordance with the nomenclature and principles described in the ICH (Q6B) guidance document "Specifications, Test Procedures, and Acceptance Criteria for Biotechnology and Biological Products." The specification limits for rhAPC Drug Substance were established based on experience with the manufacture of this product by Eli Lilly and Company and ---------- In particular, they were established based on extensive characterization of the rhAPC reference standard (Section I.E., Reference Standard), routine testing and additional characterization of clinical trial lots and full-scale consistency lots (Section I.F.2., Tabulation of Analytical Data on Drug Substance Lots), stability studies (Section I.H., Stability of the Drug Substance), and analytical methods validation (Section I.I.4., Analytical Methods Used to Control the Drug Substance).

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I.F.2.b. Certificate of Analysis for Qualification Lots

On the following pages the Certificates of Analysis for eight validation lots (-- batchs) of recombinant human Activated Protein C Drug Substance are provided. These lots of the drug substance have been manufactured at full-scale by the commercial process in the commercial facility, ----------.

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The drug substance specifications provided on the following -------Certificates of Analysis were the specifications in effect at the time of manufacture of the drug substance validation lots. The proposed drug substance specifications provided in Section I.F.1., Drug Substance Specifications and Tests, were approved by the Lilly Corporate Specification Committee on 16 November 2000. All validation lots meet the proposed drug substance specifications provided in Section I.F.1. of this application.

Reviewer's note: The certificates of analysis for these lots (----------) were presented on pages 767-790 and data for the 4th validation batch from ----------) is in amendment 125029.002.

I.F.3. Analytical Methods and Validations for rhAPC Drug Substance

Methods specific for rhAPC were developed at Eli Lilly and Company and transferred to ----------. Though ----------created a new method code for each of the transferred methods, the methods from the two testing sites are harmonized with each other. ----------analytical methods are provided with the corresponding Lilly method validations in the order specified below in Section I.I.4., Analytical Methods Used to Control the Drug Substance.

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I.G. Container Closure System

Recombinant human Activated Protein C (rhAPC) drug substance is[

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General Information on Packaging Components

(The targets and tolerances listed below are approximate, and are subject to acceptable industry standards.)

---------- tank

Drug substance contact materials:

---------- tank gasket for ---------

Nominal capacity: -------- Liters

Overall diameter ---------- inches

Manufacturer: ----------

Shipping Description

The----------drug substance is held in a ---------- and stored in a ---------- ---------- A---------- shipping container (----------)is ---------- to maintain the drug substance at ----º or less during transportation. The ---------- on the exterior surface of the vessel and to maintain temperature. The ---------- is placed in the ----------, sealed, and placed in a ----------. The ---------- is stabilized within the trailer and shipped to either Eli Lilly and Company or ---------- ----------. A temperature monitor is used to measure and record the temperatures of the inside of the ---------- during shipment. When the ---------- reaches its destination, it is removed from the ----------, and the temperature-recording

device is downloaded and the product temperature measurement is recorded. The ---------- is then placed on a ---------- at either Lilly for storage or at ---------- prior to drug product formulation.

The qualification process for bulk drug transfer assessed the temperature stratification within the ---------- from the warmest to the coldest locations under a variety of ambient temperatures. Testing continued in place with the ---------- placed in the ---------- ----------. Finally, transportation studies were init iated with the shipment of a placebo lot from -------to Lilly to ----------. Initial shipping times and interior temperatures of the ---------- were recorded and compared with results from the stationary tests. The qualification protocol was then executed for --------- bulk drug substance lots **(---------- data on pages 794-796).**

All time and temperature parameters met the qualification acceptance criteria with the exception of lot 4562 product temperature upon removal from the ---------- at Lilly. The results of the ensuing investigation into the temperature deviation showed that there was no impact on drug substance quality.

Conclusion

These studies demonstrate chemical stability and microbial control during shipping of the drug substance.

I.I.4 Analytical Methods Used to Control the Drug Substance

I.I.5 Analytical Methods Used for Drug Substance Stability Studies

A thorough description of the test methods used to control drug substance and in stability studies were contained in the appendix entitled "submethod.pdf". Also encluded are the validation studies to support the utilization of these methods. The test methods and their validation were found to be acceptable with the exception of the tests used to evaluate ---------- In particular, the test method analysis for the ----------) do not

evaluate the ----------. For more information see the 483 issued to the Eli Lilly Corporate Center on August 10, 2001.

Comments, Requests and Questions for the Sponsor:

1. Please submit the following information to the BLA:

   a. The defined lifespan for each commercial scale chromatography column and filter used in the purification of drug substance and information attesting to how the lifespan was established.

   b. Please provide information which confirms the ability to clean the filter or columns and associated equipment (i.e. injectors, etc.) over the defined lifespan.

   c. In instances where a lifespan has yet to be established, how will the commercial scale lifespan be defined and how will the ability to clean the column or filter be evaluated over the defined lifespan? What will constitute a failure in the performance of the chromatography columns and filters in these studies? What will constitute a failure in the ability to clean the columns and filters in these studies?

   d. In the case of a failure, how will the disposition of the lot(s) produced since the last passing evaluation be determined?

   e. Please provide any plans for extending the established lifespans of columns or filters.

2. Please provide information which confirms that the assays used for release testing of drug substance provide an assurance that all ---------- in rhAPC have been ----------

3. Please confirm that all drug product lots intended to be released for commercial distribution were produced by the identical validated drug substance and drug product manufacturing process.

4. The BLA contained drug substance stability data for up to -- months at --- °C and --- months at --- °C. Based on these data, an expiration dating period of --- months at ----°C can be granted. Please provide a stability protocol for FDA review. Upon review and approval of this protocol, data supporting extension of the dating period can be submitted in an annual report.

5. The drug product manufacturing section of the BLA (page 90) contains a description of the ---------- of drug product solution after a ---------- ----------. Please submit to the BLA a validation study which supports this ---------- step and includes an analysis of drug product stability following such ----------

6. Please note that -- month drug product stability data on the 10 mg clinical formulation is not adequate to support --- month expiration dating for the commercial 5 mg and 20 mg formulations. Additional real time stability data for the 5 mg and 20 mg formulations submitted in Amendment 20 is sufficient to support an 18 month expiration date. Please submit a revised drug product stability protocol that provides for placing at least one lot of both the 5 mg and 20 mg presentations on stability each year. Upon review and approval of this protocol, data supporting extension of this dating period can be submitted in the annual report.

7. Please specify the manufacturers of the -------and -------- media used in cell banking, and supply Certificates of Analysis for these media.

8. Please commit to addressing the following items and provide a time frame for completion of the commitment:

   a. Please adapt the ---------- identity test ---------- ---------- for use as a purity assay. Please implement this assay for use in ---------- and drug product release testing and in ---------- ----------. This analysis should include an evaluation of the complete ---------- ----------). Please ---------- of the reference standard ---------- which corresponds to the limit of detection for the analysis.

   b. Please perform analysis of drotrecogin alfa (activated) ---------- including ---------- content, in the drug substance and drug product stability studies to support the expiration dating. Please implement this analysis for use as a drug product release test.

   c. In the validation studies for the ------ potency test used for ---------- ---------- and drug product, the information provided regarding specificity is minimal. Since the activity is measured by the ---------- ----------), more information regarding what potential contaminants could interfere with this assay is critical. Please provide additional information on the specificity of this assay and specifically address the question of whether any given ---------- will interfere with the assay and whether any ---------- may interfere as well.

   d. Only ---------- data points are used to generate the standard curve for the ---- assay and therefore, it is not possible to be absolutely confident that

the linear part of the standard curve is being utilized in each analysis. Please utilize a standard curve in this assay which is generated from more than --------- data points.

e. Please reevaluate drug substance and drug product release specifications when sufficient commercial lots have been manufactured. Please define the number of commercial lots that will trigger such a reevaluation. Please note that the acceptance criteria should be based upon manufacturing experience.

f. Please implement routine testing of the ---------- media, and the ----------, and other parameters as appropriate. Please provide specifications for this testing.